Supplementary Methods

Cell Culture of Human Epithelial Cell Lines

Human cell lines MCF7, MDA and LNCap were kindly donated by P. Saunders (Edinburgh, UK). The human cell line A549 was kindly donated by T. Sethi (Edinburgh UK). All cell lines were cultured in Dulbecco's Modified Eagle Medium (DMEM) (Gibco, Paisley, UK) supplemented with 10% fetal calf serum, penicillin/streptomycin and L-glutamine, except for the LNCap cells, which were cultured in Roswell Park Memorial Institute media (RPMI) (Gibco, Paisley, UK). For all experiments cells were plated at semi-confluent density in 1% fetal calf serum. Chemical reagents were purchased from Sigma (Poole, UK) unless stated.

Isolation and Culture of Primary Murine Hepatocytes

Primary murine hepatocytes were isolated from C57BL/6 mice and cultured as previously described (1). In proliferation experiments, identical numbers of cells (1×10^5) were plated onto 24mm collagen-I-coated polyacrylamide gel supports and maintained in culture for 48 hours. The cells were then fixed in 4% paraformaldehyde solution and immunostaining undertaken for the Ki67 antigen. A total of 25 low power fields (x50 magnification) were counted for Ki67 positive cells for each slide (n=3).

Microscopy and Morphometric Analysis

Representative photomicrographs (x200 magnification) of cell morphology and high magnification fluorescent images were obtained using an inverted Zeiss Axiovert-200 microscope and Axiovision image acquisition software (Zeiss, Heidenheim, Germany). Cell spreading measurements were obtained by quantifying the mean projected surface area from 50 cells visualized by phase-contrast microscopy (ImageJ, National Institutes of Health, USA). Confocal microscopy was performed using an inverted Leica SP5C spectral confocal laser scanning microscope and image acquisition via LASAF software (Leica Microsystems GmbH, Wetzlar, Germany).

Preparation of Polyacrylamide Gel Supports

Polyacrylamide gels of variable stiffness were prepared on glass coverslips using a modification (2) of the method initially described by Pelham and Wang (3). Chemical reagents were purchased from Sigma (Poole, UK), unless stated. In brief, glass coverslips were soaked in 0.1N sodium hydroxide and air dried. A small aliquot of 3-aminopropyltrimethoxysilane was spread across each coverslip and the coverslips were extensively washed in distilled water and then soaked in 0.5% glutaraldehyde in PBS. The coverslips were then coated in a thin layer of gel containing a mixture of 7.5% acrylamide and 0.01-0.3% bis-acrylamide (National Diagnostics, Hessle, UK) in a HEPES buffered solution pH8. Polyacrylamide gel polymerization was promoted by the addition of 10% ammonium persulfate (APS) (1/100 volume) and TEMED (3/1000 volume). The resulting gel mixture was then pipetted onto a clean glass surface that had been treated with a hydrophobic silicon polymer (Rain-X^{TM}, SOPUS Products, Houston, USA) and inverted coverslips were then carefully lowered (treated-side down) onto the gel droplet. This procedure resulted in the formation of a uniform flat polyacrylamide coating on the pre-treated glass coverslip. The gels were then washed in 50mM HEPES and sufficient 50mM sulfosuccinimidyl 6 (4'-azido-2'-nitrophenylamido) hexanoate (Sulfo-SANPAH) (Perbio, Cramlington, UK) was added to fully cover the gel surface. The coverslips were then exposed to UV light for 5 minutes to facilitate cross-linker activation. Excess Sulfo-SANPAH was removed by extensive washing in 50mM HEPES. A thin layer of collagen-I, collagen-IV, plasma fibronectin (all from Sigma, Poole, UK) or laminin (R&D Systems, Abingdon, UK) was then crosslinked to the gels at room temperature for 90 minutes. Excess extracellular matrix protein was washed off and any remaining cross-linker blocked with 1% ethanolamine in 50mM HEPES for 30 minutes at 4^{o}C. Gels were soaked in serum free culture media overnight before plating of cells.

MTT-Assay

Identical numbers of cells were plated at non-confluent density onto 24mm polyacrylamide gel supports and cultured for 6 days in reduced serum conditions. At the end of the culture period 3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT reagent, 5mg/ml in PBS) was added and incubated with the cells for a further 90min. Cells were then washed,

culture medium removed and the formazan end-product solubilized in DMSO. The absorbance was measured at 590nm in a plate reader.

siRNA Transfection

Targeting and non-targeting siRNA pools (siGENOME Smartpool) were purchased from Dharmacon (Lafayette, USA). Each siRNA pool consisted of 4 annealed double stranded RNA oligonucleotide sequences designed to silence specific target genes or function as non-targeting (control) siRNA. Transfection of siRNA was performed using DharmaFECT 4 (Dharmacon, Lafayette, USA) according to the manufacturer's instructions. HCC cell lines were plated in DMEM media supplemented with 10% fetal calf serum and penicillin/ streptomycin (Huh7: 1.5 x 10^4cells/ cm^2 and HepG2: 3 x10^4 cells/ cm^2). The cells were then cultured overnight in standard culture conditions. In each siRNA experiment cells were transfected using DharmaFECT 4 (Huh7: 0.05µ/100µl and HepG2: 0.4µl/ 100µl) and siRNA at a final concentration of 50nM in antibiotic free media. Transfection media was replaced after 12 hours with standard antibiotic-containing media. For each experiment cells were trypsinized and transferred to polyacrylamide gel supports after 48 hours and experiments terminated at 72 hours following transfection. Gene silencing at 72 hours was assessed by western blot analysis of cell lysates using antibodies specific for FAK and β1-integrin (as previously described).

Western Blotting

Cells were harvested in lysis buffer containing 1% Triton-X-100 and a mixture of proteinase and phosphatase inhibitors. Equal amounts of proteins were separated by electrophoresis and transferred onto nitrocellulose membranes. A list of the antibodies and the concentrations at which they were employed is provided in the list below. Detection was performed using enhanced chemiluminescence (Millipore, Livingstone, UK) and bands were visualized by X-ray films (Fuji, Bedfordshire, UK). For quantification, blots from a minimum of three independent experiments were analyzed by densitometry (ImageJ, National Institute of Health, USA).

Antibodies used for Western Blot

Antibody	Source	Dilution
$p21^{cip}$	Santa Cruz Biotechnology	1:1000
$p27^{kip}$	Santa Cruz Biotechnology	1:250
HNF4alpha	Santa Cruz Biotechnology	1:200
Alpha-fetoprotein	Santa Cruz Biotechnology	1:2000
CyclinD1	Cell Signaling	1:1000
CyclinD3	Cell Signaling	1:1000
$p\text{-}ERK^{Thr202/Tyr204}$	Cell Signaling	1:2000
ERK	Cell Signaling	1:1000
$pAkt^{Ser473}$	Cell Signaling	1:2000
Akt	Cell Signaling	1:4000
$p\text{-}FAK^{Tyr397}$	Cell Signaling	1:1000
FAK	Cell Signaling	1:1000
$p\text{-}STAT3^{Tyr705}$	Cell Signaling	1:1000
STAT3	Cell Signaling	1:1000
E-Cadherin	Cell Signaling	1:1000
Smad2/3	Cell Signaling	1:1000
$p\text{-}Smad2^{Ser465/467}$	Millipore	1:1000
$p\text{-}Smad3^{Ser423/425}$	Millipore	1:2000
Albumin	DAKO	1:2,000,000
N-Cadherin	DAKO	1:500
α1-Antitrypsin	QED Bioscience	1:2000
Integrin β1	Abcam	1:1000
GAPDH	Calbiochem	1:200,000
Anti-rabbit IgG-HRP	Cell Signaling	1:2000
Anti-mouse IgG-HRP	Cell Signaling	1:2000

Santa Cruz Biotechnology, Heidelberg, Germany

Cell Signaling Technology, Danvers, MA

Millipore, Watford, UK

DAKO, Ely, UK

QED Bioscience, San Diego, USA

Calbiochem, Nottingham, UK

Abcam, Cambridge, UK

Supplemental Methods References

1. Wiegard C, Frenzel C, Herkel J, Kallen KJ, Schmitt E, Lohse AW. Murine liver antigen presenting cells control suppressor activity of CD4+CD25+ regulatory T cells. Hepatology. 2005; 42(1):193-9.

2. Engler A, Bacakova L, Newman C, Hategan A, Griffin M, Discher D. Substrate compliance versus ligand density in cell on gel responses. Biophys J 2004; 86(1 Pt 1):617-628

3. Pelham RJ, Jr., Wang Y. Cell locomotion and focal adhesions are regulated by substrate flexibility. Proc Natl Acad Sci U S A 1997; 94(25):13661-13665.